

Comment on 'Quiescence and γ H2AX in neuroblastoma are regulated by Ouabain/Na,K-ATPase': ouabain and cancer

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Sir,

Cardiac glycosides are promising cancer therapeutic agents (Lopez-Lázaro, 2007; Newman *et al*, 2008; Mijatovic *et al*, 2012). In a recent article published in the journal, Hiyoshi *et al* (2012) report that the cardiac glycoside ouabain induces quiescence in neuroblastoma cells *in vitro* and a marked reduction in tumour growth when human neuroblastoma cells are xenografted into immune-deficient mice. On the basis of these findings, the authors conclude that ouabain could be used in chemotherapies to suppress tumour growth and/or arrest cells to increase the therapeutic index in combination therapies (Hiyoshi *et al*, 2012).

It has been known for some time that rodent cells are over 100 times more resistant than human cells to the cytotoxic effects of ouabain and other cardiac glycosides (Gupta *et al*, 1986). This means that the anticancer effects induced by ouabain in mice harbouring human neuroblastoma cells (Hiyoshi *et al*, 2012) are probably due to interspecies differences in sensitivity and not to selective inhibition of tumour growth. In other words, the marked reduction in tumour growth observed when human neuroblastoma cells were xenografted into immune-deficient mice is probably caused by the ability of ouabain to kill human cells *vs* rodent cells rather than by its ability to kill cancer cells *vs* normal cells.

To further support this idea, three human cancer cell lines, three human non-malignant cell lines from the same origin, and two rodent cell lines have been exposed for 48 h to ouabain, and cell viability has then been estimated with the MTT assay. The IC₅₀ values (means \pm s.e.m.) of three independent experiments were 39.3 \pm 1.0 nm for the human MCF7 breast cancer cell line; 41.4 \pm 2.7 nm for the human MCF10 breast non-malignant cell line; 57.4 \pm 1.9 nm for the human UACC62 melanoma cell line; 60.8 \pm 11.1 nm for the non-malignant human VH10 skin fibroblast cell line; 5.3 \pm 0.4 nm for the human A549 lung cancer cell line; 29.4 \pm 4.5 nm for the human MRC5 lung non-malignant cell line; 67 000 \pm 2000 nm for the rodent VC8 cell line (deficient in homologous recombination repair); and 113 700 \pm 29 000 nm for

the rodent VC8B2 cell line (parental cell line of VC8). These results show that rodent cells are approximately 1000 times more resistant than human cells to the cytotoxic activity of ouabain and that, except for the human lung cell lines, ouabain does not inhibit the growth of the human cancer cell lines selectively. Hiyoshi *et al* (2012) reported that ouabain induced quiescence in human neuroblastoma cells at a concentration of 50 nm, and here we show that such concentration of ouabain also reduces the viability of the three human non-malignant cell lines used. The higher sensitivity of VC8 cells *vs* VC8B2 cells (Calderon-Montaño *et al*, 2012) to ouabain agree with the activation of the DNA-damage response pathway marker gamma-H2AX observed by Hiyoshi *et al* (2012) in their work.

In conclusion, the concentrations of ouabain required to induce quiescence in human neuroblastoma cells *in vitro* also reduce the viability of human non-malignant cells. In addition, the marked reduction in tumour growth observed when human neuroblastoma cells were xenografted into immune-deficient mice is probably due to the ability of ouabain to kill human cells selectively rather than by its ability to kill cancer cells selectively. These data do not support the use of ouabain in patients with neuroblastoma.

REFERENCES

Calderon-Montaño JM, Burgos-Morón E, Orta ML, Pastor N, Perez-Guerrero C, Austin CA, Mateos S, Lopez-Lázaro M (2012) Guanidine-reactive agent phenylglyoxal induces DNA damage and cancer cell death. *Pharmacol Rep* **64**: 1515–1525.

Gupta RS, Chopra A, Stetsko DK (1986) Cellular basis for the species differences in sensitivity to cardiac glycosides (digitalis). *J Cell Physiol* **127**: 197–206.

Hiyoshi H, Abdelhady S, Segerstrom L, Sveinbjornsson B, Nuriya M, Lundgren TK, Desfrere L, Miyakawa A, Yasui M, Kogner P, Johnsen JI, Andang M, Uhlen P (2012) Quiescence and gammaH2AX in

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neuroblastoma are regulated by ouabain/Na,K-ATPase. *Br J Cancer* **106**: 1807–1815.

Lopez-Lazaro M (2007) Digitoxin as an anticancer agent with selectivity for cancer cells: possible mechanisms involved. *Expert Opin Ther Targets* **11**: 1043–1053.

Mijatovic T, Dufrasne F, Kiss R (2012) Cardiotonic steroids-mediated targeting of the Na(+) /K(+) -ATPase to combat chemoresistant cancers. *Curr Med Chem* **19**: 627–646.

Newman RA, Yang P, Pawlus AD, Block KI (2008) Cardiac glycosides as novel cancer therapeutic agents. *Mol Interv* **8**: 36–49.